

CLAIMS

What is claimed is:

1. A method for binding an exogenous molecule to a binding site, wherein the binding site is located within a region of interest in cellular chromatin, wherein the 5 method comprises:
 - (a) identifying an accessible region within the region of interest;
 - (b) identifying a target site for the exogenous molecule within the accessible region; and
 - (c) introducing the exogenous molecule into the cell;
- 10 whereby the exogenous molecule binds to the binding site.
2. The method according to claim 1 wherein the cellular chromatin is in a chromosome.
3. The method according to claim 1 wherein the accessible region is a nuclelease hypersensitive region.
- 15 4. The method according to claim 1, further comprising the step of:
 - (d) testing for binding of the exogenous molecule to the binding site.
5. The method according to claim 4, wherein testing is by a method selected from the group consisting of chromatin immunoprecipitation and *in vivo* footprinting.
- 20 6. The method according to claim 1, wherein the exogenous molecule is a protein.
7. The method according to claim 6 wherein the protein performs a process selected from the group consisting of replication, recombination, integration, DNA repair, transcriptional regulation and chromatin remodeling.
- 25 8. The method according to claim 6 wherein the protein is used for detection of a target sequence.
9. The method according to claim 7, wherein the protein is a transcription factor.

10. The method according to claim 9, wherein the transcription factor is a zinc finger protein (ZFP).

11. The method according to claim 6 wherein the protein is encoded by an exogenous nucleic acid introduced into the cell.

5 12. The method according to claim 1, wherein the cell is a eukaryotic cell.

13. The method according to claim 12, wherein the cell is a plant cell.

14. The method according to claim 12, wherein the cell is a mammalian cell.

15. The method according to claim 14, wherein the cell is a human cell.

16. The method according to claim 1, wherein the binding site is in a coding 10 region.

17. The method according to claim 1, wherein the binding site is in a non-coding region.

18. The method according to claim 10, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

15 each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

19. The method according to claim 1, wherein the exogenous molecule is introduced into the cell by a method selected from the group consisting of lipid-mediated 20 gene transfer, electroporation, direct injection, particle bombardment, calcium phosphate co-precipitation, DEAE-dextran mediated transfer, and viral vector-mediated transfer.

20. The method according to claim 11 wherein the nucleic acid is introduced 25 into the cell by a method selected from the group consisting of lipid-mediated gene transfer, electroporation, direct injection, particle bombardment, calcium phosphate co-precipitation, DEAE-dextran mediated transfer ,and viral vector-mediated transfer.

21. A method for binding a ZFP transcription factor to a binding site, wherein the binding site is located within a region of interest in cellular chromatin, wherein the method comprises:

(a) identifying an accessible region within the region of interest;

5 (b) identifying a zinc finger protein (ZFP) binding sequence within the accessible region;

(c) designing a ZFP to bind to the binding sequence; and

(d) introducing the ZFP into the cell;

whereby the ZFP binds to the binding site.

10 22. The method according to claim 21 wherein the ZFP is introduced into the cell by introducing a DNA construct encoding the ZFP into the cell under conditions in which the construct expresses the ZFP.

23. The method according to claim 21 wherein the cellular chromatin is in a chromosome.

15 24. The method according to claim 21 wherein the accessible region is a nuclease hypersensitive region.

25. The method according to claim 21 further comprising the step of:

(e) testing for binding of the ZFP to the binding site.

26. The method according to claim 25 wherein testing is by a method selected 20 from the group consisting of chromatin immunoprecipitation and *in vivo* footprinting.

27. The method according to claim 21, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

25 at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

28. The method according to claim 21, wherein the ZFP is selected by phage display.

29. The method according to claim 21, wherein the ZFP is selected *in vivo*.

30. A method for identifying a binding site for an exogenous molecule, wherein the binding site is located within a region of interest in cellular chromatin, wherein the method comprises:

- 5 (a) identifying an accessible region within the region of interest; and
(b) identifying a target site for the exogenous molecule within the accessible region.

31. The method according to claim 30, further comprising the steps of:

- 10 (c) introducing the exogenous molecule into the cell; and
(d) testing for the binding of the molecule to the binding sequence.

32. The method according to claim 30 wherein the cellular chromatin is in a chromosome.

33. The method according to claim 30 wherein the accessible region is a nuclease hypersensitive region.

15 34. The method according to claim 30 wherein the exogenous molecule is a protein.

35. The method according to claim 34 wherein the protein is a transcription factor.

20 36. The method according to claim 35 wherein the transcription factor is a ZFP.

37. The method according to claim 30, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

25 at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

38. The method according to claim 31 wherein the exogenous molecule is a protein and wherein the protein is introduced into the cell by introducing a DNA

construct encoding the protein into the cell under conditions in which the construct expresses the protein.

39. The method according to claim 31 wherein testing is by a method selected from the group consisting of chromatin immunoprecipitation and *in vivo* footprinting.

5 40. A complex between an exogenous molecule and a binding site, wherein the binding site is located within a region of interest in cellular chromatin and wherein the binding site is identified according to the method of claim 30.

10 41. A complex between an exogenous molecule and a binding site, wherein the binding site is located within a region of interest in cellular chromatin and wherein the binding site is identified according to the method of claim 31.

15 42. The complex according to claim 40, wherein the exogenous molecule is a protein.

43. The complex according to claim 42, wherein the protein performs a process selected from the group consisting of replication, recombination, integration, 15 DNA repair, transcriptional regulation and chromatin remodeling.

44. The complex according to claim 42 wherein the protein is used for detection of a target sequence

45. The complex according to claim 43, wherein the protein is a transcription factor.

20 46. The complex according to claim 45, wherein the transcription factor is a zinc finger protein (ZFP).

47. The complex according to claim 40, wherein the cell is a eukaryotic cell.

48. The complex according to claim 47, wherein the cell is a plant cell.

49. The complex according to claim 47, wherein the cell is a mammalian cell.

25 50. The complex according to claim 49, wherein the cell is a human cell.

51. The complex according to claim 40, wherein the binding site is in a coding region.

52. The complex according to claim 40, wherein the binding site is in a non-coding region.

53. The complex according to claim 46, wherein the binding site comprises the sequence 5'-NNx aNy bNz c-3', wherein

5 each of (x,a), (y,b) and (z,c) is (N,N) or (G,K); and

at least one of (x,a), (y,b) and (z,c) is (G,K); wherein N is any nucleotide and K is either G or T.

54. A method for identifying a binding site, in a chromosome, for a ZFP transcription factor, wherein the chromosome is in a cell, wherein the method comprises:

10 (a) identifying a region of interest in the chromosome;

region; (b) identifying a zinc finger protein (ZFP) binding sequence within the

(c) designing a ZFP to bind to the binding sequence;

(d) introducing the ZFP into the cell; and

15 (e) testing for the binding of the ZFP to the binding sequence by chromatin immunoprecipitation;

wherein, if binding is detected in step (e), a binding site is identified.

55. A method for identifying a binding site in cellular chromatin for a ZFP transcription factor, wherein the method comprises:

20 (a) identifying a region of interest in the cellular chromatin;

region; (b) identifying a zinc finger protein (ZFP) binding sequence within the

(c) designing a ZFP to bind to the binding sequence;

(d) designing a DNA construct that encodes the ZFP of step(c);

25 (e) introducing the construct into the cell; and

(f) testing for the binding of the ZFP to the binding sequence by chromatin immunoprecipitation

wherein, if binding is detected in step (f), a binding site is identified.

56. A cell comprising a complex between an exogenous molecule and a binding site, wherein the binding site is located within a region of interest in cellular chromatin and wherein the binding site is identified according to the method of claim 30.